The National Animal Germplasm Program: Challenges and Opportunities for Poultry Genetic Resources[1]

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ABSTRACT In the United States, poultry genetic resources have consolidated because of economic pressures. Such consolidations can potentially jeopardize the poultry industry and the ability of research communities to respond to future challenges. To address the loss of genetic resources for all livestock and aquatic species, USDA established the National Animal Germplasm Program (NAGP) in 1999. Since the initiation of NAGP, population surveys have been conducted on nonindustrial chicken and turkey breeds. These surveys not only provide insight into breed status, but also serve as a benchmark for future comparisons. The survey results revealed that 20 chicken breeds and 9 turkey breeds were in various stages of being lost. The NAGP has initiated an ex situ repository for cryopreserved germplasm and tissue that already contains 59 chicken lines and 2,915 tissue samples. As the NAGP, along with its industry and university partners, continues developing the ex situ collection, there are research opportunities in cryopreserved tissue utilization and studies of genetic diversity. For cryopreserved tissues, several key research areas include improving the cryopreservation protocols for rooster and tom semen by using cryoprotectants other than glycerol and utilizing embryonic cells. Although surveys have been conducted on public research lines and rare breeds, there is a void in understanding the level of genetic diversity present in U.S. poultry populations. Therefore, an opportunity exists to perform a series of genetic diversity studies using molecular-based approaches. Such an evaluation can help clarify population differences between research lines and rare breeds and, thereby, facilitate conservation strategies. There appears to be growing consumer interest in poultry products derived from heritage breeds and/or poultry raised in nonindustrial production systems. Although the depth of such market trends is unknown, such an interest may provide an important niche for rare poultry breeds and, thereby, strengthen the genetic base.

Key words: poultry genetic resource, genetic conservation, cryopreservation, chicken, turkey

INTRODUCTION

The importance of animal genetic resources and specifically poultry genetic resources has been discussed across a range of forums (FAO, 1998; Delany 2003; Fulton and Delany, 2003; Miller, 2004) with requests for action from both public and private institutions. At both the public and private levels in the United States, there has been a reduction in the number of lines and breeds of chickens, turkeys, and Japanese quail (Pisenti et al., 2001; Blackburn et al., 2003).

As with other livestock species, economic forces drive the line/breed reductions. During the course of the last decade, the breeding industry has consolidated, resulting in a loss of many commercial and developmental lines. This process has all occurred while there has been an industry shift to greater attention on health, product quality, and animal welfare (D. Harry, Cheyenne, WY, unpublished data), which may require access to diverse genotypes. Similar losses have been observed for a second group—nonindustrial breeds (Blackburn et al., 2003)—where population numbers are small and the number of individuals raising such lines is small. The third group of poultry resources is flocks at public universities and research institutions. These populations have served a broad array of research uses over time; however, they are being reduced as institutions lose the financial and technical ability to maintain the populations (Pisenti et al., 2001). Arthur and Albers (2003) underscore this situation with the observation that the "combined losses of research and commercial populations can limit the future genetic potential of the chicken."

In general, there is recognition of the importance of the poultry genetic resources issue at technical levels, but there has been a limited response to the prospect of conserving these resources by administrative levels. This paper will

Table 1. American Livestock Breeds Conservancy classification for nonindustrial chicken and turkey breeds[1]

Species	Critical	Threatened	Watch	Recovering	Study
Chicken	20	4	10	6	15
Turkey	9	1	2	0	8

[1]Critical = fewer than 500 U.S. breeding birds with ≤5 primary breeding flocks (≥50 birds); globally endangered. Threatened = <1,000 breeding birds in the United States with ≤7 primary breeders; globally endangered. Watch = <5,000 U.S. breeding birds with ≤10 primary breeding flocks; globally endangered. Recovering = a breed once listed in another category and has exceeded the Watch category. Study = breeds that are of interest but either lack definition or lack genetic or historic documentation.

discuss the response and actions taken to date by the National Animal Germplasm Program (**NAGP**), the challenges, and the opportunities for working to secure poultry genetic resources.

NAGP

The NAGP was formed as a result of the 1990 Farm Bill passed by Congress, which calls for the Secretary of Agriculture to develop a National Genetic Resources Program that would be administered by the Agricultural Research Service. However, it was not until 1999 that the NAGP was initiated by the Agricultural Research Service and in cooperation with the Cooperative State Research, Education, and Extension Service to help assure university faculty involvement in the program. With the initiation of the NAGP, there was the formation of 6 species committees. These committees were developed to provide recommendations on developing germplasm collections, identifying lines/breeds that are in need of conservation actions, and provide an additional mechanism for taking conservation action. Committee members are from industry, universities, and government and nongovernmental organizations.

Accomplishments to Date

The NAGP has become a functioning system that acquires and stores germplasm, documents collection information, and releases material for utilization. The NAGP, in concert with its Poultry Species Committee, has addressed poultry genetic diversity issues in 3 ways: providing recommendations about populations in need of attention, assisting in prioritization of collection development, and serving as a contact point for parties interested in research and other aspects of poultry genetic resources.

Another area of NAGP activity has been collaboration with the American Livestock Breeds Conservancy, a nongovernmental organization involved in monitoring and assisting people or organizations interested in maintaining rare breeds of livestock. To date, this interaction on poultry has included collaboration on assessing the population status of rare breeds of turkeys and chickens. The results of those surveys can be found in Blackburn et al. (2003). The American Livestock Breeds Conservancy categories for the condition of nonindustrial chicken and turkey breeds are given in Table 1. Although long-term trend data are not available for these populations, the survey indicated that one-third to one-half of the breeds are classified as being in

critical condition. This situation may be further aggravated because of the limited number of hatcheries that sell rare breeds of poultry and a lack of insight about the existing diversity of the breeding populations that are sold by the hatcheries.

A third area of NAGP activity is the development of cryopreserved collections of germplasm. These collections are being constructed to fulfill several needs. The primary purpose of the reserve is to provide a secure backup for national security or situations where industry or breeders need access to diverse genetic resources to solve genetically influenced problems. A secondary purpose is to provide the research and/or breeding community with a source of material that could be used in developing new lines or as a source of DNA for genomic studies.

In addition to developing species committees, NAGP developed a repository as a component of the National Center for Genetic Resources Preservation. As part of this development, it became apparent early in the program's development that there would be a need for different collection categories to serve various purposes. As a result, 4 collection categories were created: core, evaluation, working, and restricted, the definitions of each are given by Blackburn (2004). In brief, the core collection should contain enough germplasm for 150% of regeneration needs for the population of interest. Evaluation and working collections have varying amounts of germplasm to be utilized by industry or research entities. Restricted collections consist of germplasm that have intellectual property protections in place.

Collection goals for chicken and turkey breeds have been established by FAO (1998) at 6,544 and 9,816 straws (0.25 mL) of frozen semen, respectively. Their estimate is conservatively set to 200% of regeneration and DNA study needs. The NAGP has set a core collection goal of 150% of regeneration needs (no other uses); therefore, the core collection would be set at 1,633 and 3,681 straws (0.5 mL) for chicken and turkey breeds, respectively [note straw volume differences between the Food and Agriculture Organization of the United Nations (**FAO**) and NAGP]. However, there are many unique subpopulations within a breed that exist in industry and public institutions. Therefore, an additional set of core collection goals is suggested for single-gene mutation lines (45 straws) and quantitative trait research lines (150 straws).

Collection Development

In general, an opportunistic approach has been used to develop the initial cryopreserved collection. For poultry,

Table 2. Cryopreserved semen and blood by chicken breed

Breed	Lines	Animals	Semen	Blood
New Hampshire	3	21	81	135
Rhode Island Red	1	6	7	51
White Leghorn	51	413	1,678	565
White Plymouth Rock	2	6	355	—
Crossbred	2	5	11	32
Total	59	451	2,132	783

the primary focus has been the collection of public research lines. Table 2 provides an overview of semen and blood collected and stored by chicken breed. Within these breeds, 59 research lines have been collected. These lines represent mutant, congenic, random-bred controls, and quantitatively selected lines. Acquiring germplasm or tissue has been accomplished in varying ways. For example, NAGP staff have traveled to the target populations' location and collected and frozen germplasm over several days. A second approach has been to ship roosters of targeted populations to Fort Collins, Colorado and collect the roosters until sufficient quantities of germplasm were collected and frozen. The third approach was for the institution maintaining the population to collect and freeze blood and semen samples.

Because of the breeding industry's concerns for their competitive position, no germplasm or tissue has been added to the repository from this source. To address concerns by the industry, a restricted category was developed to provide protection for material having intellectual property rights.

CHALLENGES AND OPPORTUNITIES

The NAGP's challenges and opportunities are multifaceted. A strategic challenge is to develop a collection of germplasm and tissue that enables the repository to respond to a range of issues that the community is currently facing and uses or needs that future users might face. Collections should be acquired and developed so that users have the ability to respond to 1) national or industry crises, 2) industry needs (including the breeding industry and rare or minor breed fanciers), and 3) university and research populations. In building the repository collection, attention must be focused on genetic diversity, the logistics of acquiring those resources, and the technical aspects of cryopreserving selected tissues. Although NAGP does not have the resources to maintain live animal populations, there are ways that NAGP can be of assistance, such as exploring genetic distances between populations, developing mating strategies, and evaluating pedigrees.

Genetic Resources

Understanding the extent of avian genetic diversity is key for developing germplasm and tissue collections as well as for developing strategies to conserve and exploit those resources. From a national perspective, the question may be asked as to how well avian genetic resources are

understood? It would seem that for lines within public institutions and large commercial breeding firms, the understanding is considerable. However, for rare breeds or lines of chickens and turkeys, there are knowledge voids at the production, pedigree and population structure, and molecular levels.

Even though our understanding of commercial populations is extensive, concern has been expressed by several researchers that those genetic resources may be approaching biological limits and have a relatively narrow genetic base (Fairfull and Gowe, 1986; Cahaner, 1990; Delany, 2003). However, others state that there is sufficient genetic diversity in broiler and layer populations (Dunnington et al., 1994). In a study of 52 chicken populations, it was determined that broiler and layer lines (comprising 40% of the populations) had near average heterozygosity and alleles per locus for the tested microsatellites (Hillel et al., 2003). Although commercial populations were present in their study, the relevance to US commercial populations is unclear. Within the United States, Zhou and Lammont (1999) constructed a phylogenic tree on 23 inbred Leghorn-derived research lines and concluded that the populations could be grouped into 4 clusters. However, it is important to note that these researchers also detected the presence of line-specific alleles.

By some accounts, industry's pedigreed lines have an effective population size of 80 to 100 head, which is larger than the FAO (1998) recommendation of 50 head and is within the 50- to 100-head range that Meuwissen (1999) suggests is critical for maintenance of a viable population. Compared with the previously mentioned benchmarks, these populations may not be in immediate risk, but they are within the range to warrant monitoring and taking precautionary steps to maintain genetic diversity.

If it is assumed that there is sufficient genetic diversity and that mutation rates are sufficient to introduce new variation, the situation still exists that, with increased selection pressure, a number of genetic abnormalities may occur that can negatively impact productivity. For example, negative correlations have been shown between increased growth rate and immune response, increased weight gain and static or decreased bone structure to support weight increases, as well as the occurrence of ascites (Dunningham, 1990; Figueiredo et al., 1998; Sewalem et al., 1998). Given these types of issues and viewing such problems from a repository vantage point, it seems prudent to position a collection to offer a range of diverse germplasm to assist industry to mitigate them.

With the first draft of the chicken genome complete, it becomes evident that genetic resources are enabling technologies. As further mapping and understanding of the chicken genome proceed, there will be opportunities to increase the utilization of chicken research lines. For example, Kuhnlein et al. (2003) identified several approaches to "implicate" DNA polymorphisms: selection response in closed breeding populations, trait association studies in closed populations, and segregation analysis within families. All 3 of these approaches will need diverse genetic populations to explore and elucidate DNA polymorphisms. Therefore, the existing poultry populations could play a very significant role in furthering the understanding of the chicken genome and its functional genomics.

Technical Aspects

There is a wide range of technical aspects that have proven to be challenges in the development of a chicken gene bank. However, there are potential solutions to these challenges, and, therefore, all chicken lines can and should be cryopreserved to retain the maximum diversity. Furthermore, with the exception of low postthaw semen viability/fertilization rates, there is no documented impediment to reconstituting selected industry lines, random-bred control lines, inbred research lines, or mutant lines.

Acquisition of germplasm or other tissue from identified poultry populations is the primary challenge. Although collection and shipment of blood is easily accomplished, semen collection is not. There are 2 impediments: one is the availability of people to collect and process semen, and the second is the ability to hold, ship, and cryopreserve rooster semen. Given the relatively low volume of semen collected per ejaculate compared with other species, development of chicken collections has substantially larger collection costs. From 2 different collection exercises by our laboratory, the average per-straw collection cost was $12.00 (range = $2.47 to $21.25). As a comparison, off-site sheep collections average $2.53 per straw (range = $1.17 to $3.90). The average cost per straw is highly dependent on the number of roosters available for collection and the speed at which the samples can be collected and frozen.

Biological limitations to cryopreserving semen are the use of glycerol as a cryoprotectant, because of its contraceptive effects (Hammerstedt and Graham, 1992), and the relatively low ejaculate volume of roosters. In addition, challenges exist to optimize cryopreservation protocols in terms of the number of cells needed per insemination, the volume of insemination dosage, and freeze rates (Donoghue and Wishart, 2000; Terada et al., 1989).

Work in The Netherlands and France provides a potential solution to rooster preservation. Chalah et al. (1999) followed by Woelders et al. (2006) reported fertility rates from cryopreserved rooster semen of 88% (vs. 93% for the control, $P > 0.05$) when dimethylacetamide was used. Dimethylacetamide had significantly higher fertility than samples treated with glycerol. Even with their reported increase in fertility, Chalah et al. (1999) report that only one-third to one-half of the initial population of spermatozoa

survived the deleterious effects of cryopreservation. However, similar orders of magnitude for cell survival have been observed in our laboratory with swine and small ruminants.

There has been discussion that breed and line differences are sufficiently great as to limit the ability to cryopreserve viable semen. However, there have been no conclusive studies concerning this issue. Research by Bacon et al. (1986), using inbred and specialized chicken strains, indicated that such differences are minor and should not be an impediment to developing cryopreserved stores. At NAGP, postthaw evaluation was performed between 2 lines of White Leghorn and 2 lines of White Plymouth Rock roosters using computer-assisted sperm analysis and flow cytometry. No differences between breeds, line within breed, rooster within line and breed, and collection date were detected (Table 3). The similarity of the percentage of motility and motion straightness would suggest no breed or line differences. However, large but nonsignificant mean differences for viability and cell area were detected.

Another question often raised concerns the longevity of rooster semen when stored in liquid nitrogen. Although a definitive answer is not available, we have compared samples frozen by Bacon et al. (1986) to samples frozen in 2005 (Table 4). For motility, straightness, and path velocity, there were no significant differences between years. Even though different protocols were used for cryopreservation, the results indicate that there is no deterioration of rooster sperm once it has been successfully cryopreserved.

Embryonic Cells. A drawback to securing chicken genetic resources is the lack of ability to capture mitochondrial DNA because of the large size of the egg. Also, although semen can be utilized to regenerate populations, it requires 3 to 5 backcrosses to obtain the desired levels in the reconstituted population. An exciting potential solution for resolving these issues is the utilization of primordial germ cells (**PGC**) or blastodermal cells. Petitte et al. (1990) injected stage X embryo blastodermal cells into a host egg and obtained a rooster that was both a somatic and germ cell chimera. The efficacy of such early work was low (<1%), but has increased by compromising the host embryo via radiation or chemical approaches (Song, 2003). Naito et al. (1994) demonstrated that PGC could be cryopreserved with 94% postthaw viability using 10% dimethyl-sulfoxide. Such high postthaw viability is an encouraging aspect of utilizing PGC, given the relatively low fertility rates generally achieved with rooster sperm frozen when glycerol is used.

Although work to date utilizing PGC is encouraging, further evaluation on several fronts is needed before knowing whether utilizing PGC is a viable approach to genetic conservation. These include exploration in the areas of cryopreservation protocols, optimal number of cells to inject into the host egg, mechanisms to lower host egg PGC levels, and the effects of the interaction that may occur when the donor and host are different sexes (Kagami et al., 1997).

The principal measure of success of using PGC will be the integration of the donor's cells into the recipient's gonads in sufficient quantities that line reconstitution can be done

Table 3. Least squares means for postthaw semen characteristics of 2 breeds of chicken

Breed and line	Motility	Straightness	Viability	Cell area
Leghorn; high antibody	47.2[a]	66.2[a]	69.2[a]	14.3[a]
Leghorn; low antibody	49.3[a]	66.5[a]	68.6[a]	12.5[a]
Plymouth Rock; high gain	49.5[a]	66.0[a]	53.5[a]	14.8[a]
Plymouth Rock; low gain	59.3[a]	66.5[a]	70.2[a]	14.4[a]

[a]Values in columns with different superscript letters differ ($P < 0.05$).

with ease and at a relatively rapid rate. Significant increases in the rate of transmission have occurred since the <1% of Petitte et al. (1990) to 3.1% by Chang et al. (1997) to 9% by Park et al. (2003). Additional increases in transmission rate would certainly be desirable and would increase the efficacy of using PGC. However, given a germ cell chimeric hen at the 10% transmission level, 1 out of 10 eggs will be from the donor genotype and, thereby, will reduce the number of years needed to regenerate a population.

Success of the PGC approach also implies being able to differentiate between the resulting donor genotype and the recipient genotype through genetic testing. This issue becomes moot if a sterile recipient line of poultry is developed. Such lines have been previously developed for different purposes (Thorne and Sheldon, 1991; Thorne et al., 1997). However, it should be noted that these lines had low viability, and the work is still in its infancy.

Institutional and Breeder Challenges

Across U.S. teaching and research institutions, there has been a contraction of resources for the maintenance of live animal populations. This contraction is due to reduced financial resources allocated to animal sciences as a whole. As a result, livestock programs have a reduced presence at many land-grant institutions. As an example, among livestock species, dairy cattle have experienced the greatest decrease; there is only one remaining genetically unique population of Holstein cattle, which is located at the University of Minnesota. Fulton and Delany (2003) and Miller et al. (2004) have brought this matter to the attention of a broad spectrum of the scientific community. However, these alerts have not translated into support for or enhancement of genetically unique populations. This case seems to mirror the global situation. Globally, awareness has been raised; however, funding for genetic conservation is either below perceived levels of need or has not materialized despite the discussions at the FAO. Commission on Genetic Resources for Food and Agriculture.

Further analyses are necessary to more effectively contextualize and publicize the seriousness of the national and global situations. These include the level of animal line utilization by researcher(s), timeframe(s) needed to create or recreate animal lines, the use of research results generated by unique animal populations by industry or the human medical community, and the economic valuation of such resources (Gollin and Evenson, 2003).

For major and minor or rare breeders, implementation of market-oriented breeding programs will continue to drive selection strategies and the choice of genetic resources. Consumers have shown a growing interest and concern for animal welfare and health issues. To what degree these factors will impact animal genetic resources is undetermined at this time. However, the American Livestock Breed Association reports (Don Bixby, personal communication) that in 2004, approximately 50,000 heritage breed turkeys were sold. In its assessment, this volume represents a substantial sales increase and has stimulated an even greater production of noncommercial turkey breeds. If such market activity increases further, there could be a broadening of genetic resources utilized to meet an array of consumer preferences.

FUTURE ACTIONS

Conservation of poultry genetic resources is in the early stage of development, especially when compared with the U.S. plant genetic resources system, which has been in place since the 1940s. Development of cryopreserved stores and maintenance of live research populations are underway and will continue as major efforts. There are opportunities to enhance understanding the status of genetic resources and mechanisms for the conservation of in situ and ex situ material. Additional efforts are needed to enhance, conserve, and understand the genetic diversity of rare and minor chicken, turkey, duck, and goose breeds. This will require a significant input because of the large number of breeders, geographic distribution, and numerous conservation methodologies that will have to be applied to conserve such populations. Although large breeding firms may believe their populations to be biosecure, events in the United Kingdom and southeast Asia should raise the issue of de-

Table 4. Least squares means for postthaw comparison of rooster semen frozen in 1985 and 2005 using computer-assisted sperm analysis

Year	n	Motility	Straightness	Path velocity
1984	10	24.6[a]	73.3[a]	45.0[a]
2005	12	15.3[a]	76.0[a]	37.2[a]

[a]Values in columns with different superscript letters differ ($P < 0.05$).

veloping additional cryopreserved stores of poultry germplasm as an added level of security.

In 2004, the NAGP Poultry Committee determined that some of their immediate actions should include establishment of standardized procedures for collecting and freezing poultry semen so that more institutions can collect and process germplasm, more investment in developing better protocols because of the difficulty in cryopreserving poultry germplasm, prioritization of lines for collection, and development of database templates more specific to poultry.

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